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Patterns of genetic diversity in Mediterranean supralittoral amphipods (Crustacea, Amphipoda)

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Abstract

Allozymic variation at 26 loci was investigated in 9 populations of *Talitrus saltator* and 4 populations of *Talorchestia deshayesii* from different Mediterranean area. Within *T. saltator* a considerable amount of genetic differentiation was found, allowing the recognition of three genetically different groups of populations (Tyrrhenian, Adriatic and Eastern-Mediterranean) with a mean $D_{Nei'78}$ value of 0.334. With regard to *T. deshayesii*, the population from the Maltese Archipelago was the most differentiated with a D_{mean} of 0.228 compared to populations believed to be conspecific on morphological grounds. These results are compared with allozymic data from our laboratory on 5 genera and 10 species of supralittoral amphipods. The observed pattern of genetic variation could be the result of the intrinsic ecological features of the species as well as environmental differences among the collecting sites.

Key words: Amphipoda, allozymes, genetic divergence, speciation, environmental stress

1. Introduction

Talitrid amphipods are particularly suitable organisms for the evaluation of levels of genetic differentiation on both micro- and macro- geographic scales. Presently, a quite detailed knowledge of the pattern of genetic structuring of several species of supralittoral talitrids has been obtained at the scale of the whole Mediterranean and adjacent Atlantic areas (De Matthaeis *et al.* 1994; 1995; 1996; 1998; 2000; Bulnheim, Scholl 1986; Bulnheim, Schwenzer 1999). Results from these studies have shown how supralittoral amphipods might be considered as good model organisms to investigate the genetic aspects of allopatric modes of

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speciation. In particular, according to Allmon (1992), speciation by allopatry may be divided into three stages: formation, persistence and differentiation of isolated populations. Data on the spatial pattern of genetic diversity in *Talitrus saltator* (De Matthaeis *et al.* 1995) may well be used to assign a given genetic distance to each of the above steps. This is of particular importance if supralittoral amphipods are considered for use as genetic indicators of the quality of the beaches where they dwell. In fact, due the abundance of their populations, a high proportion of the energy flow passes through them, such that every habitat perturbation is expected to have an effect on the genetic structure of these organisms. That environmental stresses may cause variation in the genetic structure of natural populations has now been demonstrated experimentally in several studies (see the review Hummel, Patarnello 1994), althought the exact mechanism of the genetic effects of environmental disturbance is far from being fully understood. To analyse the possible role of environmental alterations in shaping the genetic structure of natural populations of a given species, a good knowledge of the degree of genetic structuring of the species on a wide geographic area is necessary, in order to discriminate between local factors and variation on a macro-geographic scale.

In the present paper we report data on the degree of genetic differentiation and variability in several populations of *T. saltator* and *Talorchestia deshayesii*. For *T. saltator* we have compared some new populations with ones belonging to the geographic groups recognised in De Matthaeis *et al.* (1995) in order to confirm the pattern of genetic differentiation of this species at a Mediterranean scale. *T. deshayesii* was studied to obtain a comparable data set on a species closely related to *T. saltator* and with similar ecology. For both species the sampling strategy was designed to compare populations from anthropogenically-impacted beaches with unimpacted controls. This will complement the results of Scapini *et al.* (1995) who have demonstrated a positive correlation between coastal stability, heterozygosity levels and orientation capability (a peculiar behavioural trait of sandhopper) in several Mediterranean populations of *T. saltator*.

2. Material and methods

Nine populations of *T. saltator* and four populations of *T. deshayesii* were studied for genetic polymorphisms. Collecting sites and sample sizes are given in Fig. 1. Animals were transported live to the laboratory and then stored at -80°C. The following 15 enzymatic proteins, coded by 26 presumptive gene loci, were tested in each populations (the scored loci are given in parentheses): Acph (*Acph-1; Acph-2; Acph-3*); Ada (*Ada*); Ao (*Ao*); Aph (*Aph-1; Aph-2*); Ca (*Ca-1; Ca-2*); Est (*Est-1; Est-2*); Got (*Got-1; Got-2*); Hk (*Hk*); Lap (*Lap-1; Lap-2; Lap-3*); Ldh (*Ldh*); Mpi (*Mpi*); Pep (*Pep-1; Pep-2; Pep-3*); Pgm (*Pgm*); Phi (*Phi*); To (*To-1; To-2*). Details on protein codes and electrophoretic procedures are given elsewhere (De Matthaeis *et al.* 1994; 1995). The degree of genetic differentiation within and between species was assessed on the basis of Nei's genetic distance *D* (Nei 1978). The neighbour-joining (*NJ*; Saitou, Nei 1987) method was used to infer the pattern of genetic relationships between the populations studied. Robustness of each node was assessed by producing 1000 bootstrapped genetic distance matrices. Levels of genetic variability were calculated by using the following parameters: H_e (expected mean heterozygosity under Hardy-Weinberg equilibrium); H_o (observed mean heterozygosity), *P* (proportion of polymorphic loci according to the 1% criterion) and *A* (mean number of alleles *per locus*). Wright's F-statistics (1965) were used to characterise overall genetic subdivision within each species. Wright's parameters were calculated using the estimators of Weir, Cockerham (1984) where f and θ correspond to F_{is} and F_{st} in Wright's notation. f and θ were computed at single loci and over all loci: f was used to assess possible deviations from Hardy-Weinberg equilibrium, while θ was used to quantify subdivision among populations. Variance of f and θ was evaluated through the resampling techniques of jackknifing and bootstrapping. Details on these techniques are given in De

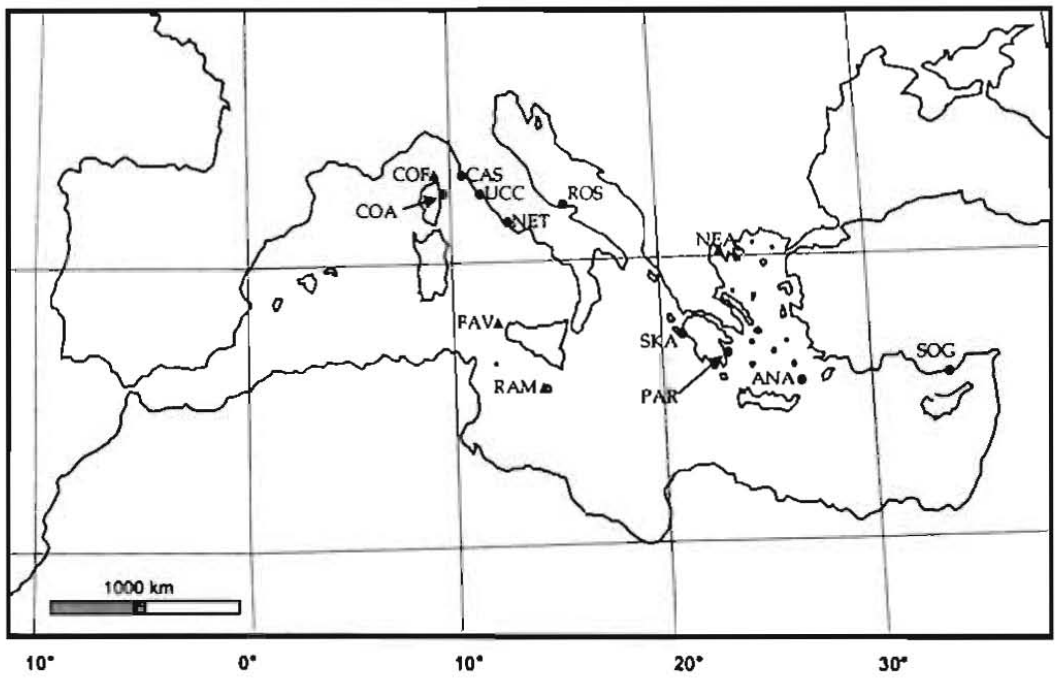

Fig. 1. Sampling localities of *Talitrus saltator* (circles) and *Talorchestia deshayesii* (triangles): COA – Anghione; CAS – Castiglion della Pescaia; UCC – Uccellina; NET – Torre Astura; ROS – Rodi Garganico; SKA – Skafidia; PAR – Paralio Astros; ANA – Anafi; SOG – Soguksu; COF – Saint Florent; FAV – Favignana; RAM – Gozo; NEA – Nea Plagia

Matthaeis *et al.* (1998). One thousand replicates each of bootstrapping and jackknifing were produced. The θ values per pairwise comparisons within each species were also calculated. An indirect estimate of gene flow was calculated according to the following relation $Nm = 1/4((1/F_{st})-1)$ (Wright 1931). The *Nm* values were obtained both over all loci and for pairwise comparisons. Data analyses were carried out by using the following packages: Biosys-1 (Swofford, Selander 1981), F-stat (Goudet 1995), Genepop (Raymond, Rousset 1995) and Phylip 3.5 (Felsenstein 1995).

3. Results

Allele frequencies at the 27 scored loci and variability estimates of the populations studied are given in Table I. 23 loci were monomorphic in all populations and species (*Acph-1; Acph-2; Acph-3; Ada; Ao; Aph-1; Aph-2; Ca-1; Ca-2; Est-1; Est-2; Got-1; Got-2; Hk; Lap-1; Lap-2; Lap-3; Ldh; Pep-1; Pep-2; Pep-3; To-1; To-2*). *Acph-2, Aph-1; Aph-2; Lap-1, Pep-1* and *Pep-2* were fixed for the same allele in all populations; these loci are not reported in Table I.

Table I. Allele frequencies and variability estimates in study talitrid populations. N is the sample size. letters indicate alleles at different loci

Locus/	*T. salator*									*T. deshayesii*			
Pop	COA	CAS	UCC	NET	ROS	SKA	PAR	ANA	SOG	COF	FAV	RAM	NEA
Acph-1													
N	10	10	14	11	15	14	18	15	18	18	17	23	16
A	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1.000	1 000	1.000	0 000	1 000
B	0 000	0 000	0 000	0 000	0 000	0.000	0 000	0 000	0 000	0.000	0.000	1 000	0 000
Acph-3													
N	14	23	14	11	48	14	18	15	45	19	18	23	11
A	0 000	0 000	0.000	0 000	0 000	0 000	0 000	0 000	0.000	0 000	0 000	1 000	0 000
B	0 000	0 000	0 000	0 000	0 000	1.000	1 000	1 000	1 000	0.000	0.000	0.000	0 000
C	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0.000	0 000	0 000	0 000
D	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
E	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	0.000	1 000
Ada													
N	13	24	16	11	35	11	17	15	24	17	12	17	10
A	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
B	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000
Ao-1													
N	15	17	16	12	17	17	17	15	18	14	19	18	15
A	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
B	0 000	0 000	0 000	0 000	0.000	1 000	1 000	1.000	1 000	0 000	0 000	0 000	0 000
C	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
D	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Cu-1													
N	14	24	15	12	39	18	17	15	26	11	12	18	13
A	1 000	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
B	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Cu-2													
N	14	24	15	12	39	18	17	15	26	11	12	18	13
A	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
B	0 000	0 000	0 000	0 000	0.000	0 000	0 000	0 000	0 000	0 000	1 000	0 000	0 000
C	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0.000	0 000
D	0 000	0 000	0 000	0 000	0.000	1 000	1.000	1.000	1.000	0.000	0 000	0 000	0 000
E	0 000	0 000	0 000	0 000	0 000	0 000	0.000	0 000	0.000	1 000	0 000	1 000	1 000
Est-1													
N	14	16	13	12	25	13	18	15	34	16	18	15	10
A	1 000	1 000	1.000	1 000	0 000	1 000	1 000	1 000	1 000	0 000	0 000	0 0 0	0 000
B	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
Est-2													
N	14	15	16	12	36	17	18	15	31	11	12	15	15
A	1 000	1 000	1 000	1 000	0 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
B	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Got-1													
N	18	24	15	12	44	18	18	15	37	20	12	11	15
A	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000
B	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
C	0 000	0 000	0 000	0 000	0 000	0 000	0.000	0 000	0 000	1 000	0 000	1 000	1 000
D	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	0 000	0 000
Got-2													
N	14	12	15	12	20	10	18	15	12	18	12	11	10
A	0 000	0 000	0 000	0.000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
B	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000
Hk													
N	14	28	19	12	65	16	18	15	58	29	11	20	14
A	1 000	1 000	1 000	1.000	1 000	1.000	1 000	1 000	1 000	0 000	0 000	0 000	0 000
B	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000

Locus /Pop.	*T. salator*									*T. deshayesii*			
	COA	CAS	UCC	NET	ROS	SKA	PAR	ANA	SOG	COF	FAV	RAM	NEA
Lap-3													
N	15	24	14	11	32	10	17	15	21	15	11	10	15
A	0.000	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
B	1.000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	0 000	0 000	0 000	0 000
Ldh													
N	14	16	12	12	36	17	18	15	39	13	11	10	18
A	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1.000	1 000	0 000	0 000	0 000	0 000
B	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	1 000	1 000	1 000
Mpi													
N	13	23	13	12	42	18	18	15	44	19	11	11	16
A	0 654	0 000	0 000	0 250	0 833	0 000	0 000	0 000	0 239	0 000	0 000	0 000	0 000
B	0 346	0 870	1 000	0 750	0 167	1 000	1.000	1 000	0 761	0 000	0 000	1 000	0 000
C	0 000	0 130	0 000	0 000	0 000	0.000	0.000	0 000	0 000	0.895	0 818	0 000	1 000
D	0.000	0 000	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 105	0 182	0 000	0 000
Pep-3													
N	14	27	13	12	46	18	18	15	38	24	12	16	15
A	0.000	0.000	0 000	0.000	0 000	0 000	0.000	0.000	0.000	0.000	0 000	0 000	1.000
B	0.000	0.000	0 000	0 000	0 000	1 000	1 000	1.000	1.000	0 000	0 000	0.000	0 000
C	0.000	0.000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000
D	1.000	1.000	1 000	1 000	0 000	0 000	0 000	0 000	0.000	0 000	1 000	1 000	0 000
Pgm													
N	15	17	10	12	34	13	18	15	37	15	12	17	7
A	0.000	0.000	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	1 000	0 000
B	0.000	0.000	0.100	0.083	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
C	1 000	1.000	0 900	0 917	1 000	1 000	1 000	1 000	0 919	1 000	0 917	0 000	0 857
D	0.000	0.000	0.000	0.000	0.000	0.000	0 000	0 000	0 081	0 000	0 083	0 000	0 143
Pk													
N	13	23	6	10	39	17	18	15	38	20	12	10	15
A	0.000	0.109	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0 000
B	1.000	0.326	0.000	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0 000	0.000	0.000
C	0.000	0.565	0.833	0.500	1.000	1.000	1.000	1.000	1.000	0.850	1.000	0 000	0 933
D	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0 000	0 067
Tw-1													
N	10	19	12	12	14	18	18	15	15	13	11	15	11
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1 000	1.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0 000
Tw-2													
N													
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0 000	0 000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1 000	1 000
A	1.0± .0	1.1± .1	1.1± .1	1.1± .1	1.0± .0	1.0± .0	1.0± .0	1.0± .0	1.1± .1	1 1± 1	1 1± .1	1 0± 0	1 1± 1
*P**	3.8	7.7	7.7	11.5	3.8	0.0	0.0	0.0	7 7	7.7	7 7	0.0	7 7
Ho	.009±.009	.017±.017	.021±.015	.029±.024	.005±.005	.000± .000	.000± .000	.000± .000	.018± .013	.012± .009	.020± .015	.000± .000	.016± .012
*He***	.018±.018	.051±.024	.019±.013	.041±.025	.011±.011	.000± .000	.000± .000	.000± .000	.020± .015	.017± .012	.018± .013	.000± .000	.015± .011

*criterion of 0.99

**Unbiased estimate (see Nei 1978).

Genetic variation within *T. saltator*

Acph-3, Ao-1, Ca-2, Est-1 and *Est-2* were diagnostic between Tyrrhenian populations and Adriatic ones; *Acph-3, Ao-1; Ca-1* and *Got-1* discriminate the Tyrrhenian group from an East- Mediterranean group which includes

populations from the Ionian slope of the Peloponnese, Aegean islands and Turkish coast. Finally, the following loci are diagnostic markers between the Adriatic population (ROS) and those belonging to the East-Mediterranean group: *Acph-3, Ao-1, Ca-1, Ca-2, Est-1, Est-2, Got-1, Pep-3*. Estimates of genetic variability (Table I) generally indicate a low level of polymorphism: H_o ranges from 0.000 (SKA; PAR; ANA) to 0.029 (NET), while H_e ranged between 0.000 and 0.041 for the same populations. The results of F-statistics analyses at single locus and over all loci are reported in Table II: f was low and not significant for two of the three polymorphic loci scored in the study populations (*Pgm*: f= –0.060 and *Phi*: f= 0.111), while f was positive and significant at the locus *Mpi* (f = 0.512, $p \leq 0.01$) and over all loci (0.319, $p \leq 0.01$) indicating a departure from the Hardy-Weinberg equilibrium due to heterozygote deficiency. The θ was low and not significant only at the locus *Pgm* (0.041); positive and significant values were obtained at the *Mpi* and *Phi* loci and over all loci (0.479; 0.627; 0.921 respectively, $p \leq 0.01$). The high values of θ indicated that the genetic diversity within *T. saltator* is mainly due to interpopulation differentiation. The θ values for pairwise comparisons ranged between 0.000 between SKA, PAR and ANA and 0.969 for the comparison ROS *vs* SKA and PAR. Indirect estimate of gene flow (*Nm*) values were very high among SKA, PAR and ANA and 0.008 between ROS *vs* SKA and PAR. The θ and *Nm* values, as well as genetic distances D, are reported in Table III. D ranged from 0.000 among SKA, PAR and ANA to 0.358 (UCC *vs* ROS).

Table II. The θ and *Nm* values at single locus and over all loci for all populations. Jackknife and bootstrap values(95% CI) are also reported

Locus	*T. saltator*				*T. deshayesii*			
	θ	θ jackknife (SD)	f	f jackknife (SD)	θ	θ jackknife (SD)	f	f jackknife (SD)
Mpi	0.479*	0.576 (0.255)	0.512*	0.467 (0.185)	0.735*	1.082 (0.573)	-0.166	-0.173 (0.103)
Pgm	0.041	0.036 (0.019)	-0.060	-0.060 (0.008)	0.889*	1.452 (0.656)	-0.087	-0.086 (0.045)
Phi	0.627*	0.665 (0.325)	0.111	0.251 (0.276)	0.740*	1.089 (0.607)	0.445	0.690 (0.425)
Over all loci	0.921*	0.925 (0.054)	0.319*	0.438 (0.279)	0.927*	0.928 (0.042)	0.069	0.050 (0.282)
Bootstr. over all loci (95% CI)	0.796 0.988	-	-0.060 0.512	-	0.838 0.982	-	-0.166 0.455	-

*$p \leq 0.01$.

Table III. Below diagonal genetic distances D (Nei, 1978) for all study populations; above diagonal θ (upper) and Nm (lower) values within the two species

	T. saltator									*T. deshayesii*			
Pop.	COA	CAS	UCC	NET	ROS	SKA	PAR	ANA	SOG	COF	FAV	RAM	NEA
COA	*****	0.508	0.734	0.321	0.960	0.960	0.960	0.953	0.931	-	-	-	-
		0.241	0.091	0.527	0.010	0.010	0.010	0.012	0.018				
CAS	0.029	*****	0.108	0.034	0.941	0.922	0.908	0.899	0.090	-	-	-	-
			2.062	6.959	0.015	0.021	0.025	0.028	0.026				
UCC	0.051	0.004	*****	0.205	0.962	0.974	0.962	0.953	0.926	-	-	-	-
				0.969	0.010	0.006	0.010	0.012	0.020				
NET	0.015	0.002	0.009	*****	0.943	0.923	0.907	0.890	0.907	-	-	-	-
					0.015	0.021	0.025	0.031	0.025				
ROS	0.375	0.363	0.358	0.355	*****	0.969	0.969	0.967	0.947	-	-	-	-
						0.008	0.008	0.008	0.014				
SKA	0.340	0.275	0.266	0.284	0.353	*****	0.000	0.000	0.098	-	-	-	-
							∞	∞	2.283				
PAR	0.340	0.275	0.266	0.284	0.353	0.000	*****	0.000	0.092	-	-	-	-
								∞	2.441				
ANA	0.340	0.275	0.266	0.284	0.353	0.000	0.000	*****	0.067	-	-	-	-
									3.457				
SOG	0.330	0.280	0.272	0.284	0.339	0.002	0.002	0.002	*****	-	-	-	-
COF	1.043	0.972	0.974	0.994	0.860	0.865	0.865	0.865	0.863	*****	0.859	0.950	0.699
											0.041	0.013	0.107
FAV	0.946	0.873	0.873	0.892	0.949	0.859	0.859	0.859	0.855	0.126	*****	0.964	0.857
												0.009	0.042
RAM	1.127	1.048	1.051	1.068	1.283	1.061	1.061	1.061	1.078	0.254	0.305	*****	0.974
													0.007
NEA	1.060	0.982	0.983	1.004	0.964	0.872	0.872	0.872	0.867	0.041	0.126	0.255	*****

Within geographic groups we obtained mean D values of 0.018 for the Tyrrhenian populations and 0.001 for the Eastern-Mediterranean ones. D increases respectively to 0.291 for the comparison between Tyrrhenian and Eastern-Mediterranean groups, to 0.349 between Adriatic and Eastern-Mediterranean populations and to 0.362 between Tyrrhenian and Adriatic populations.

Genetic variation within *T. deshayesii*

An appreciable degree of genetic differentiation was found within *T. deshayesii*. The RAM population possessed fixed alternative alleles with respect to conspecific populations at the following loci: *Acph-1, Acph-3, Mpi, Pgm* and *Phi*. A certain degree of genetic differentiation was also shown by FAV, since in this population *Ca-2* and *Got-1* loci carried diagnostic markers; finally COF and NEA were characterised by being fixed for alternative allele at the locus *Pep-3* (Table I). With regard to genetic variability, all estimates clearly indicate that RAM is deprived of genetic polymorphism (H_o, H_e and P being equal to 0.000). The mean observed and Hardy-Weinberg expected heterozygosity was 0.016 for the other populations (Table I). The f was low (with the exception of the locus *Phi*) and always not significant at any single locus and over all loci, indicating that the populations studied were basically in Hardy-Weinberg equilibrium (Table II). The θ was always positive and significant at the *Mpi, Phi, Pgm* loci and over all loci (0.735; 0.889; 0.740; 0.927, $p \leq 0.01$) indicating that the main component of the genetic variation within this species is due to differences between populations (Table II). The θ values for pairwise comparisons ranged from 0.699 (COF *vs* NEA) to 0.974 (RAM *vs* NEA). Relative *Nm* values were equal to 0.107 and 0.007 for the same comparisons, indicating a general lack of gene flow (Table III). A wide range of D values was found (Table III). It ranged from 0.041 (COF *vs* NEA) to 0.305 (FAV *vs* RAM). RAM was, on average, the most differentiated population, with a D_{mean} of 0.228 compared to populations believed to be conspecific on morphological grounds (Ruffo, pers. Comm.).

Genetic variation between *T. saltator* and *T. deshayesii*

The following loci were diagnostic between the two genera: *Acph-3, Ada, Ao, Ca-2, Got-1, Got-2, Hk, Lap-2, Lap-3, Ldh, To-1* and *To-2*. D values for between- genera comparisons ranged from 0.860 (ROS *vs* COF) to 1.283 (ROS *vs* RAM) with a D_{mean} of 0.960. Figure 2 shows the majority rule consensus tree obtained by NJ bootstrap analyses, which resulted generally robust in terms of bootstrap values. Within *T. saltator* there is a clear geographic pattern: ROS, the earliest branch and the most differentiated population, joins both the Eastern- Mediterranean and Tyrrhenian groups. The latter appeared relatively structured with Corsican population split from those

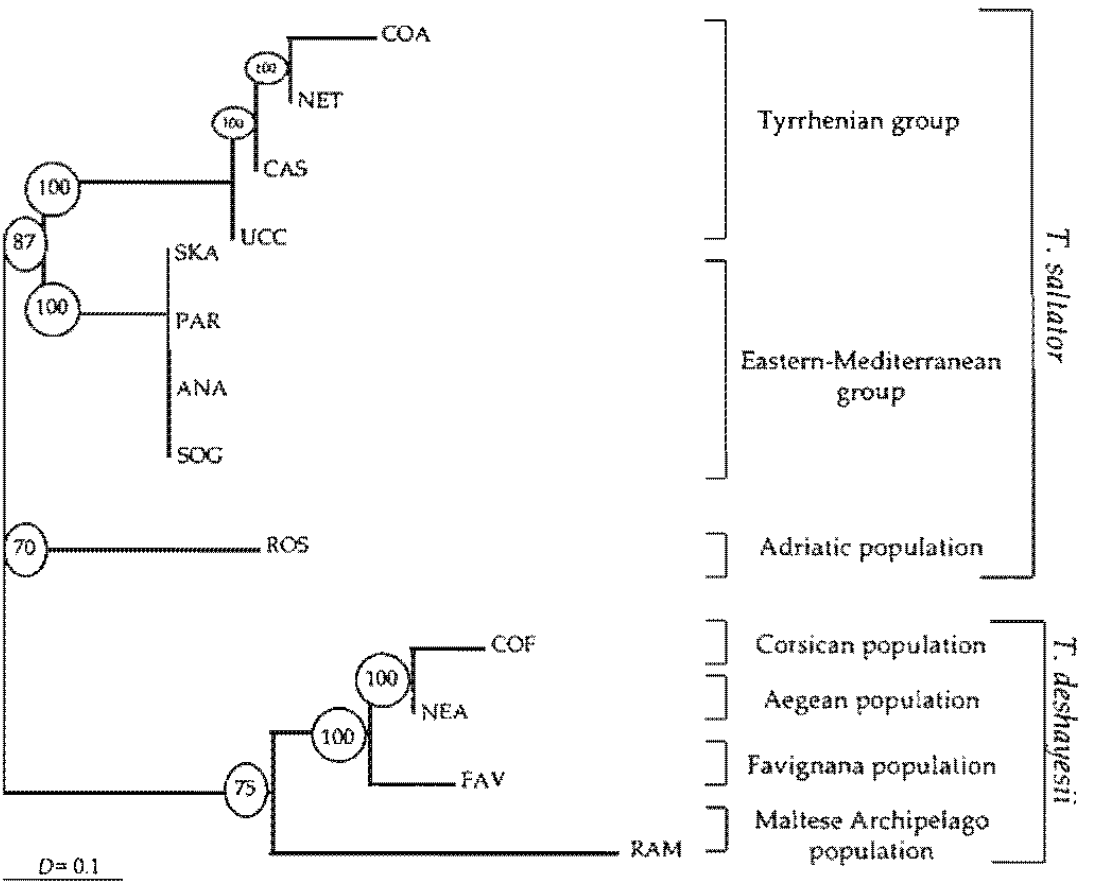

Fig. 2. Majority rule consensus tree obtained by NJ bootstrap analyses. Circled nodes include bootstrap percentages of 1000 replications

collected along the Tyrrhenian coasts. On the contrary, Eastern-Mediterranean populations were quite genetically homogeneous. Our data were not sufficient to produce a clear geographic pattern within *T. deshayesii*: RAM was the most differentiated population, but the two most geographically distant populations (COF and NEA) were also characterised by the lowest degree of genetic differentiation

Discussion

Biochemical systematics

During the last three decades enzymes have proved to be exeellent diagnostic characters, frequently revealing levels of genetie differentiation often not detected by morphological studies (Avise 1994). On the basis of the huge literature available, it has been possible to define ranges of allozyme differentiation

between taxa at various stages of evolutionary divergence (Ayala 1983). Generally, the genetic distance increases with increasing taxonomic distance, however the electrophoretically detectable differences are not accumulated at the same rate across different lineages. A within-taxon standardization should avoid possible misleading interpretations of genetic distance data, providing a threshold for each stage of evolutionary divergence. In our laboratory supralittoral amphipods have been the object of long term research projects for several years, yielding data on 5 genera and 10 species for a total of 789 population pairwise comparisons. This information is presented in Table IV as a framework against which to compare the data presented in this paper.

Levels of genetic differentiation among the geographic groups of *T. saltator* here considered matched those reported in Table IV, confirming the strong pattern of genetic structuring of this species at the scale of the Mediterranean. This is supported by the analysis of θ and Nm values, both over all loci and for pairwise comparisons, which clearly indicated a general lack of gene flow among the populations studied. *T. saltator* can be considered as a species complex, confirming the morphological observations of Ruffo (1936) and the experimental results of Scapini, Fasinella (1990), which demonstrated that secondary contact of geographical races either results in some degree of infertility among offspring or else there is no hybridisation.

For *T. deshayesii* also, the genetic data indicate a strong pattern of genetic differentiation, with high θ values over all loci and pairwise comparisons and low levels of gene flow. This pattern is mostly due to the high degree of genetic divergence detected between the population from the Maltese archipelago (RAM) and the others considered here. On average, this population was differentiated from the other conspecific populations considered at a D_{mean} value of 0.228. This value is fairly above the highest value obtained for between geographic group comparisons in the genus *Talorchestia* (Table IV). This result is not completely consistent with previous work on *T. deshayesii*, that showed a negligible amount of genetic divergence between northern European, Atlantic and Mediterranean populations (Bulnheim, Scholl 1986; De Matthaeis *et al.* 1994). A possible explanation for this discrepancy might be the particular conditions at the sampling site of the RAM population. Here, individuals were sampled more than 25 meters up the shore from the water level and from a depth of 0.5 m in the sand. Since in talitrids long distance dispersal is mainly passive and occasional and occurs through animals attached to drifting wrack or wood, it is reasonable to hypothesize that individuals of the RAM population have a low probability of being routinely carried away from the shore by sea storms. In such a scenario genetic differences can easily arise, since the low rate of dispersal cannot counteract the action of stochastic processes.

As a general rule, different levels of genetic differentiation are observed: high between geographically separated populations of *Talitrus* and *Talorchestia* (sandhoppers) and low between analogous populations in *Orchestia* and

Table IV. Mean and range of genetic distance D (Nei, 1978) for 10 species of supralittoral amphipods at different stages of evolutionary divergence

Taxa	Number of pairwise comparisons, mean $D_{Nei'78}$ values and ranges									
	within geographic groups			between geographic group			between morphologically differentiated species		between genera	
Talitrus saltator[a]	141	0.038	0.000–0.250	252	0.368	0.206–0.596		-		
Orchestia (5 species)[b]	177	0.048	0.000–0.052	159	0.036	0.006–0.083	10	0.728	0.399–1.192	
Platorchestia platensis[b]	6	0.003	0.000–0.008	4	0.010	0.005–0.015		-		
Talorchestia (2 species)[c]	1		0.047	10	0.119	0.008–0.203	2	0.888	0.877–0.900	
Talitrus, *Orchestia* (5 species); *Platorchestia*; *Talorchestia* (2 species); *Transorchestia*[c]	-		-	-		-		-	27	1.010 0.776–1.495

Data from:
[a]De Matthaeis *et al.* (1995);
[b]De Matthaeis *et al.* (1996; 1998; 2000 and unpublished data);
[c]De Matthaeis *et al.* (1994 and unpublished data); Seveso *et al.* (1998).

Platorchestia (beach-fleas) (Table IV). Beach-fleas are common on all kinds of shores and are particularly abundant in beach wracks close to the water line. In contrast, sandhoppers occurr exclusively at or above the high-water marks of sandy beaches. These ecological differences lead to different probabilities of dispersal via drifting materials, on the different kinds of shores colonised by these organisms.

Finally, the mean value of genetic distance (0.960) between *Talitrus* and *Talorchestia* falls within the range for between-genera comparisons reported in Table IV and is slightly lower than the average value obtained from 27 pairwise intra-generic comparisons.

Talitrid amphipods as indicators of environmental stresses

Several studies have demonstrated a non-random association between levels of genetic variability and temporal and spatial patterns of environmental variations (Ayala *et al.* 1974; Sbordoni 1980). Environmental stress may cause variation in the genetic structure of natural populations. Korol *et al.* (1996) have mathematically demonstrated that cyclical changes of the environmental optimum cannot help in polymorphism maintenance. Over the years, several attempts have been made to relate interspecific genetic variation to environmental factors, as a first step in using allozymic markers as indicators of the degree of human-induced alterations in a given environment (Hummel, Patarnello 1994). Beaches are fragile, marginal ecosystems, characterised by low-energy flow with supralittoral amphipods playing a central role in ecosystem dynamics. In *T. saltator* a positive correlation has been demonstrated between stability of the coastline, orientation behaviour and heterozygosity level (Scapini *et al.* 1995). In particular, the correlation between coastal stability and mean heterozygosity suggests that temporal stability may have a positive effect on genetic variability. At the same time, mean heterozygosity was shown to be significantly correlated with seaward orientation for laboratory-born individuals, where learning could not affect orientation, while no such correlation was found for wild-caught individuals, for which experience may have affected orientation. In the present study, the average degree of heterozygosity was not found to be very high, according to results previously achieved on both species (De Matthaeis *et al.* 1994), however, some differences may be highlighted. In *T. saltator* an appreciable difference between Tyrrhenian and Eastern-Mediterranean populations is shown by the degree of observed mean heterozygosity (0.019 *vs* 0.004). This result does not seem to be affected by the sample size, since UCC and NET (the most polymorphic populations) have mean sample sizes per locus smaller or equal to those of ROS or SKA, characterised by a virtual zero level of heterozygosity. This is good agreement with the results of Scapini *et al.* (1995) and seems to support the hypothesis that populations living on unimpacted beaches with stable or accreting dunes (such as UCC and NET) are more genetically polymorphic than

those of impacted beaches (ROS) with eroding dunes or a receding coastline. Ramla l-Hamra is a sandy beach on the northern coast of the island of Gozo. It is one of very few sandy beaches in the Maltese Islands where only some 2.5% of the coastline is not rocky (Anderson, Schembri 1989). The present beach at Ramla consists of modern beach sands backed by wind-blown dunes and raised beach deposits; this site is one of only three localities in the Maltese Islands that still supports a sand dune ecosystem.

The beach is visited by thousands of locals and tourists, especially during the summer months. Many types of activities that take place in the area have direct or indirect impacts on the biota of the beach, including the amphipods. Examples of such activities include vehicular access onto the beach and dunes, disturbance of the sand-binding vegetation, removal of sand, as well as barbecues and camping on the sand.

The beach at Ramla is characterised by the extreme poverty of sandy beach fauna, including such characteristic and abundant (on European-Mediterranean mainland beaches) in talitrid amphipods. In a study of the beach biota, Sammut (1995) only collected a total of 267 individuals representing 25 species of fauna from a combined volume of 1.1758 m^3 of sand, by sieving. Only three specimens of *T. deshayesii* were collected, all in summer and approximately 30–60 m away from sea level, and at a depth in the sand of 20–30 cm. Mediterranean beaches are known to be characterised by few species and low species density (Dexter 1989), however, even in the Mediterranean context, Ramla still seems to be anomalously species poor. The low population densities of *T. deshayesii* might be explained in terms of physical edaphic properties, such as compacting of the sand due to trampling, or may be due to some other constraint, such as lack of nutrients or human disturbance.

Among the *T. deshayesii* populations studied, that from RAM was characterised by absence of genetic polymorphism, while the other populations displayed similar levels of genetic heterozygosity that are comparable to those found by De Matthaeis *et al.* (1994) in several other populations of this species. Once again, this may be the result of the particular different conditions at the collecting sites.

If these results will be confirmed on a larger scale allozyme heterozygosity could be used as a predictive tool in environmental management.

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